A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition Chemie

GDCh

www.angewandte.org

Accepted Article

Title: Potent lymphatic translocation and spatial control over innate immune activation by polymer-lipid amphiphile conjugates of small molecule TLR7/8 agonists

Authors: Jana De Vrieze, Benoit Louage, Kim De Swarte, Zifu Zhong, Ruben De Coen, Simon Van Herck, Lutz Nuhn, Camilla Kaas Frich, Alexander Zelikin, Stefan Lienenklaus, Niek Sanders, Sunil David, Bart Lambrecht, and Bruno de Geest

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201905687
Angew. Chem. 10.1002/ange.201905687

Link to VoR: http://dx.doi.org/10.1002/anie.201905687
http://dx.doi.org/10.1002/ange.201905687

WILEY-VCH

10.1002/anie.201905687

WILEY-VCH

COMMUNICATION

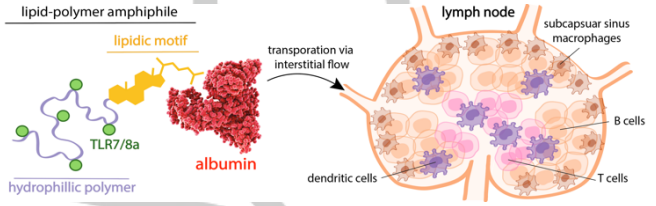
Potent lymphatic translocation and spatial control over innate immune activation by polymer-lipid amphiphile conjugates of small molecule TLR7/8 agonists

Jana De Vrieze,[a] Benoit Louage,[a] Kim De Swarte,[b] Zifu Zhong,[c] Ruben De Coen,[a] Simon Van Herck,[a] Lutz Nuhn,[d] Camilla Kaas Frich,[e] Alexander N. Zelikin,[e] Stefan Lienenklaus,[f] Niek N. Sanders,[c] Bart N. Lambrecht,[b] Sunil A. David,[g] Bruno G. De Geest*[a]

Abstract: Uncontrolled systemic inflammatory immune triggering has hampered clinical translation of several classes of small molecule immune-modulators such as imidazoquinoline TLR7/8 agonists for vaccine design and cancer immunotherapy. Taking advantage of the inherent serum protein-binding property of lipid motifs and their tendency to accumulate in lymphoid tissue we design amphiphilic lipid-polymer conjugates that confer suppression of systemic inflammation but provoke potent lymph node immune activation. In our work we provide a rational base for the design of lipid-polymer amphiphiles for optimized lymphoid targeting.

Spatial control over the activity of small molecule immune-stimulatory compounds is key in the successful design of potent vaccines and cancer immune-therapeutics.[1–3] For example, Toll like receptors (TLRs) 7 and 8, localized on the endosomal membrane of innate immune cells, recognize viral RNA as natural ligand and triggering causes robust type I interferon responses that are strong mediators of anti-viral and anti-tumoral immune responses.[4,5] Potent small molecule agonists of TLR7/8 (TLR7/8a) have been discovered, but like many other small molecule drugs, are prone to rapid distribution throughout the body, thereby losing site-specific activity and causing systemic inflammation. [6–8]

The aim of this work was to devise a simple strategy that offers spatial control of the immune-stimulatory activity of small molecule TLR7/8a. Previously, we and others have demonstrated that nanoparticle-conjugation is a potent method to restrict TLR agonistic activity,[9–12] upon local subcutaneous administration, to the site of administration and to draining lymphatic tissue where TLR7/8 triggering provokes robust activation of innate immune



Scheme 1| Concept of lymph node targeting lipid-polymer amphiphile conjugates. A hydrophilic polymer backbone provides good water-solubility of the amphiphile construct. The lipid moiety has high affinity for albumin which, following local administration, mediates transportation via the interstitial flow to lymphoid tissue. Small molecule immunostimulatory drugs (i.e. TLR7/8a) are linked to the polymer backbone and upon arrival in lymphoid tissue provoke potent innate immune activation.

cell subsets. These papers emphasize the key contribution of the colloidal nature of the resulting conjugate to achieve lymph node focused immune-stimulation.[13] Indeed, fully hydrophilic TLR7/8a conjugates that exist in unimeric single chain form in solution, exhibit a dramatic reduction in lymphatic translocation. Systems based on self-assembly of random co-polymers, block copolymers or crosslinked nanogels have an inherent lack of control over macromolecular and/or supramolecular structure. Moreover, non-degradable by-products are prone to long-term accumulation, which limit the potential for clinical translation. These considerations foster new efforts towards the design of more simple TLR7/8a carrier systems that are equally or more potent in lymphatic translocation and innate immune activation.

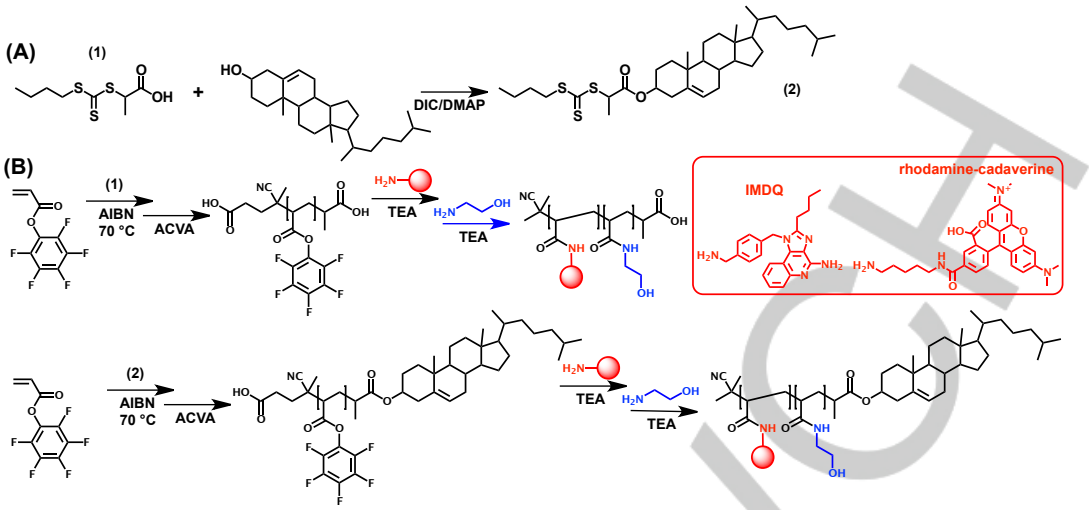
Lipid-mediated non-covalent albumin binding has proven a powerful strategy for lymphoid delivery of imaging agents[15] and polymer-drug conjugates[17] and is also known to prologue drug circulation in the blood stream [16] upon systemic administration. Recent work from the Irvine group reported that lipid amphiphiles containing CpG as a macromolecular TLR9 agonist, show, upon subcutaneous injection, robust lymphatic translocation by hitch hiking onto albumin molecules in the interstitial flow from the injection site to draining lymphoid tissue.[14] In addition, cholesterol-conjugates have also been reported to bind to other serum proteins, such as lipoproteins, too. [18] Translation of this approach to small molecule TLR7/8a (i.e. design of lipid-TLR7/8a conjugates) has proven successful to reduce unwanted systemic immune-stimulation but fails to provoke immune-stimulation in draining lymphoid tissue, due to inherent solubility issues.[19,20]

Here, we circumvent these issues by designing lipid-polymer amphiphiles consisting of a lipid motif as hydrophobic part and a hydrophilic polymer connected to the lipid moiety at one of its chain ends. Onto the polymer backbone multiple TLR7/8a motifs

[a] Jana De Vrieze, Dr. Benoit Louage, Dr; Ruben De Coen, Dr. Simon Van Herck, Prof.Dr. Bruno G. De Geest. Department of Pharmaceutics, Ghent University, Ghent, Belgium
E-mail: br.degeest@ugent.be
[b] Kim De Swarte, Prof.Dr. Bart N. Lambrecht. IRC VIB-UGent, Zwijnaarde, Belgium
[c] Zifu Zhong, Prof.Dr. Niek N. Sanders. Department of Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium.
[d] Dr. Lutz Nuhn. Max Planck Institute for Polymer Research, Mainz, Germany
[e] Dr. Camilla Kaas Frich, Prof.Dr. Alexander N. Zelikin. Department of Chemistry, Aarhus University, Aarhus C, Denmark.
[f] Dr. Stefan Lienenklaus. Institute for Laboratory Animal Science and Institute of Immunology, Hanover Medical School, Hanover, Germany
[g] Prof.Dr. Sunil A. David. Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA.

Supporting information for this article is given via a link at the end of the document.

10.1002/anie.201905687



Scheme 2| Synthesis of lipid-polymer amphiphile conjugates. (A) A cholesterol-functionalized chain transfer agent (CTA) for RAFT polymerization is synthesized by esterification. **(B)** RAFT polymerization of pentafluorophenyl acrylate yields a reactive ester backbone that is by post-polymerization modification substituted with an amine-containing dye or with the imidazoquinoline TLR7/8 agonist IMDQ followed by reacting remaining PFP-esters with 2-aminoethanol to generate a hydrophilic polymer backbone.

Table 1| Characterization of cholesterol-polyPFPA and polyPFPA.

CTA	M/CTA/ AIBN	conv. (%)	DP	M_n^{Theor} (kDa)	M_n^{SEC} (kDa)	Đ
cholesterol-PABTC	120/1/0.2	90	108	26.3	12.9	1.22
PABTC	120/1/0.2	89	107	25.7	13.5	1.15

are conjugated. This concept is schematically shown in **Scheme 1**. In first instance, we investigated whether amphiphilic lipid-polymer conjugates allow for a more efficient lymphatic translocation compared to a non-amphiphilic hydrophilic polymer. Hereto, we synthesized a cholesterol-functionalized chain transfer agent (CTA) for reversible addition-fragmentation chain transfer (RAFT) polymerization[21] by esterification of 2-propanoic acid butyl trithiocarbonate (PABTC) and cholesterol according to **Scheme 2**. The cholesterol-CTA was used together with unmodified PABTC (as a control) for RAFT polymerization of pentafluorophenyl acrylate (PFPA)[22] (degree of polymerization (DP) of 100) to generate a reactive ester polymer backbone that can later on be used for conjugation of TLR7/8 agonist molecules, as well as for fluorescent labelling (**Scheme 2**). Polymer analysis by NMR spectroscopy and size exclusion chromatography (SEC) is summarized in **Table 1**. Overall, the measured dispersity was relatively low, indicating good control over polymerization. The trithiocarbonate RAFT end group was removed by treating the polymer with an excess of 4,4'-azobis(4-cyanovaleric acid) (ACVA) to avoid later on issues on disulfide formation.[23] In a next step, the polymers were fluorescently labelled by nucleophilic substitution with rhodamine cadaverine targeting a degree of substitution (DS: number of modified repeating units per 100) of 1. Finally, a hydrophilic polymer backbone was generated by reacting remaining PFP-ester motifs with an excess of 2-aminoethanol (**Scheme 2**).

Subsequently, these polymers were tested for their interaction with cells *in vitro*, thereby probing for the influence of the cholesterol moiety. These experiments were performed on murine RAW macrophages as model innate immune cells and conducted at 4 °C to block endocytosis and at 37 °C to mimic normal physiological conditions. Flow cytometry (**Figure 1A**) indicated a clear dose-response binding of the cholesterol-polymer amphiphiles at both temperatures (**A1**: 4 °C, **A2**: 37 °C), with minimal binding of the non-amphiphile control polymer. Interestingly, also at 4 °C where endocytosis is blocked, significant cellular association of the cholesterol-polymer amphiphiles was observed. To shed light on the underlying interaction, confocal microscopy was performed (**Figure 1B**), revealing that pulsing cells with cholesterol-polymer amphiphiles at 4 °C results in the conjugates' red fluorescence being exclusively located at the cell membrane. These findings suggest that cholesterol-polymer conjugates anchor to the phospholipid cell membrane through hydrophobic interaction. These findings are in line with previous reports on the ability of cholesterol analogues to drive cell surface anchoring of macromolecular species.[24–26] When cells are pulsed at 37 °C, confocal microscopy depicts a punctuated pattern, indicating that the cholesterol-polymer amphiphiles become endocytosed into vesicular compartments (i.e. endosomes and lysosomes). Interestingly, control polymers that lack the cholesterol moiety do not show any interaction with the cell membrane and show very low cell uptake at physiological temperature, at least within the tested experimental window. To further explore the influence of amphiphile design on cell membrane anchoring ability, we synthesized two other cholesterol-polymer amphiphiles with a DP of respectively 30 and 60. Flow cytometry analysis (**Figure S14**) indicated that polymers with a shorter chain length did less efficiently anchor to cell membranes at 4 °C. Hence cholesterol-polymer amphiphiles with a DP of 100 were selected for further evaluation.

10.1002/anie.201905687

WILEY-VCH

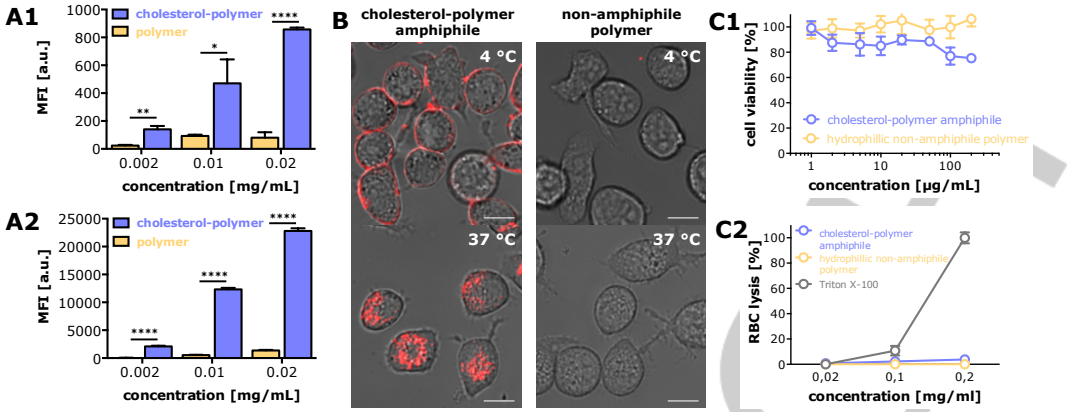


Figure 1| *In vitro* cell interaction of lipid-polymer amphiphile conjugates. (**A**) Flow cytometry analysis of murine RAW macrophages pulsed at 4°C (**A1**) and 37 °C (**A2**) with amphiphile conjugates and control. t-test: *:p<0,1; **:p<0,01; ****:p<0,0001 (n=3, mean + SD). (**B**) Corresponding confocal microscopy images depicting an overlay of the red fluorescence channel (note that conjugates were labeled with rhodamine) and the transmitted light channel. Scale bar represents 15 micron. Cytotoxic (**C1**) (based on MTT assay, n=3) and hemolytic activity (n=3) (**C2**) of amphiphile conjugates and controls.

Importantly, cytotoxicity measured by MTT assay (**Figure 1C1**) and membrane-destabilizing activity measured by chicken red blood cells (RBC) lysis assay (**Figure 1C2**), showed no significant detrimental effect for both cholesterol-polymer amphiphiles and hydrophilic non-amphiphile polymers. Taken together, these findings highlight the translational potential of the cholesterol-polymer amphiphiles having much more benign properties than an e.g. an ionic amphiphile surfactant such as Triton-X.

Subsequently, we tested lymphatic translocation of the lipid-polymer conjugates. Hereto mice were injected subcutaneously into the footpad, followed by dissection of the draining popliteal lymph node (a schematic overview of the mouse lymphoid system is shown in **Figure S15**) at 4 h, 24 h and 96 h post injection. FACS analysis shows a dramatic effect of the presence of the cholesterol motif on lymphatic translocation (**Figure 2A**). Whereas non-amphiphilic polymers show relatively low lymphatic translocation above baseline (cfr. **Figure S16** for a corresponding histogram), amphiphilicity promotes highly efficient lymphatic translocation with very high percentages of dendritic cells (DCs), B cells and macrophages in the draining lymph node being positive for the cholesterol-polymer conjugates (**Figure 2B**). These data are confirmed by confocal microscopy (**Figure 2C**) which shows massive accumulation of polymer in the subcapsular sinus and underlying B cell follicles as soon as 4 h post injection and lasting for at least up to 4 days. Based on the observation that amphiphilic cholesterol-polymer conjugates reach draining lymphoid tissue that early post-injection [9,14,27] it is reasonable to conclude that these compounds exhibit highly efficient passive translocation to lymphoid tissue and do not require uptake by immune cells at the site of injection for subsequent well-mediated lymphatic transportation.

Lipid amphiphiles have been reported to exhibit affinity to albumin, and lipoproteins, and hence promote lymphatic translocation to lymphoid tissue by hitch hiking the interstitial albumin flow.[14,16,17] To verify whether this hypothesis also holds true for our cholesterol-polymer conjugate design we conducted a series of experiments to investigate albumin binding. Biolayer interferometry (BLI) using albumin-coated sensors showed a high affinity of the cholesterol-polymer amphiphiles whereas low affinity was found for the non-amphiphile control (**Figure 3A**).

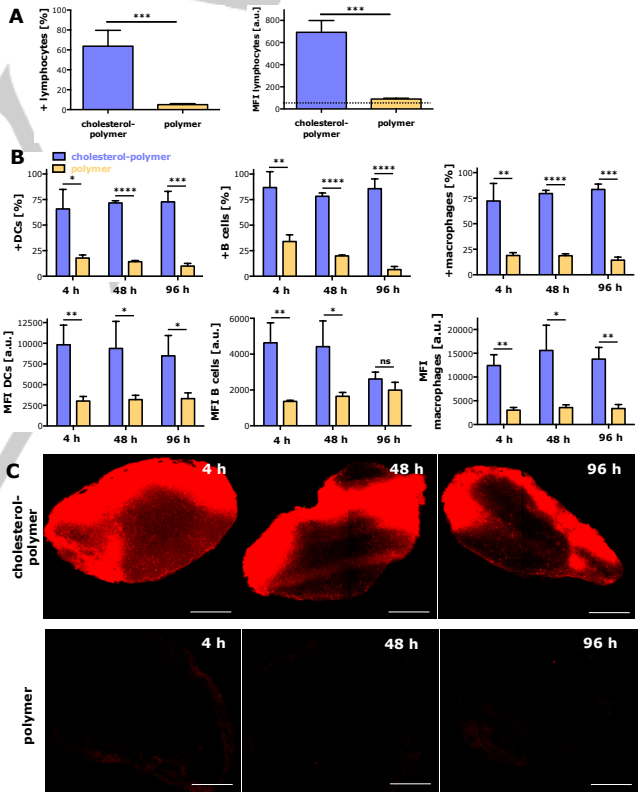


Figure 2| *In vivo* behavior of lipid-polymer amphiphile conjugates. (A) Flow cytometry analysis on total lymphocyte population in the draining popliteal lymph node of mice, 24h post-injection in the footpad. t-test; ***:p<0,001 (n=3, mean + SD). Dotted line in panel A depicts the baseline value of the untreated control. **(B)** Flow cytometry analysis of conjugate uptake by immune cell subsets at different times post-injection. t-test; ****:p<0,0001; ***:p<0,001; **:p<0,01; *:p<0,1 (n=3, mean + SD). **(C)** Corresponding confocal microscopy images of popliteal lymph nodes (tissue sections) at different time points post-injection in the footpad. Scale bar represents 200 micron.

10.1002/anie.201905687

Aqueous SEC on mixtures of albumin and polymer at different ratio (**Figure 3B**) showed a pronounced decrease in retention time with increasing ratio of cholesterol-polymer to albumin, thereby indicating both species to interact with each other. By contrast, hydrophilic non-amphiphilic polymers had no influence on the retention time of albumin. Taken together, these data proof that the cholesterol-polymer amphiphiles have very strong albumin-binding properties, which supports the hypothesis that albumin-binding is a potent mediator of lymphoid translocation and is not only capable of translocating of relatively small constructs such as amphiphile dyes, peptides and oligonucleotides,[14] but also larger macromolecules such as those used in this work.

To further explore the role of the lipid motif on albumin binding and lymphatic translocation, we synthesized polymers containing a monoalkyl (i.e. steraryl) lipid and dialkyl (i.e. 1,2-distearoyl-sn-glycero-3- phosphoethanolamine (DSPE)) lipid moiety at their chain end respectively (**Scheme S1** in Supporting Information). *In vitro* cell uptake and *in vivo* lymphatic transportation experiments indicated these amphiphiles to be sub-optimal relative to cholesterol-based amphiphiles (**Figure S17-18** in Supporting Information). Albumin-binding studies showed that monoalkyl amphiphiles did bind albumin, but to a lesser extent than cholesterol amphiphiles. Confocal microscopy at 4 °C also did not indicate cell membrane anchoring of the monoalkyl amphiphiles, thereby highlighting the influence of cholesterol-mediated membrane anchoring on increasing cellular uptake of the amphiphile conjugates. Dialkyl amphiphiles did strongly bind to albumin (**Figure S19**). However, due to the increased hydrophobicity of the DSPE motif, relative to a monoalkyl or cholesterol moiety, the resulting amphiphiles showed large aggregates (**Figure S19**), which is likely responsible for reduced cellular uptake and lymphatic translocation. These findings highlight the subtle balance between albumin binding and colloidal stability and its influence on tissue mobility upon subcutaneous administration. Indeed, whereas we and others have previously shown that colloidally stable nanoparticles with sub 200 nm dimension and inert surface can exert high mobility towards lymphoid tissue,[9,11,12,28] even for colloids with particularly low albumin-binding properties,[13,28] more hydrophobic compounds that are prone to aggregate formation. The latter mainly form a depot at the site of injection,[12,20] which has been associated with lower efficacy when used as delivery vehicle for immunostimulatory drugs. Although we demonstrate for the particular case in this paper that cholesterol-polymer amphiphiles exhibit superior *in vitro* and *in vivo* performance, a window of opportunity remains to further engineer a dialkyl amphiphile system with improved aqueous solubility and likely also superior lymphatic translocation capacity, especially in view of the findings by the Irvine group regarding dialkyl-oligonucleotide conjugates.[14]

Having proven the ability of the cholesterol polymer system to efficiently mediate cell uptake *in vitro* and *in vivo*, we used this approach for the design of amphiphilic cholesterol-polymer TLR7/8a conjugates. Hereto, the polyPFPA backbone was substituted with the small molecule imidazoquinoline TLR7/8a IMDQ,[7,29] according to **Scheme 2**. HPLC analysis showed that

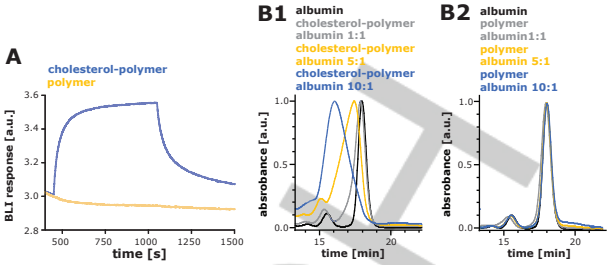


Figure 3| Albumin binding assessment. (A) Bio-layer interferometry sensorgrams of affinity towards albumin. Albumin-coated sensors were immersed at time point 500s into cholesterol-polymer or polymer solutions to measure adsorption and immersed into PBS solution at time point 1100s to measure desorption. **(B)** SEC elugrams of albumin and mixture of albumin and cholesterol-polymer **(B1)** or polymer **(B2)** at different ratios.

upon extensive dialysis the final product did not contain any free soluble IMDQ (**Figure S20**) and UV-vis spectrophotometry analysis (**Figure S21**) was used to quantify the IMDQ load. We also confirmed by BLI that IMDQ-conjugation did not impair the albumin-binding ability of the conjugate (**Figure S22**).The ability of these conjugates to trigger TLR activation was tested using the RAW-Blue NF-kB activation *in vitro* reporter cell assay and (**Figure 4A**) showed that although a significant loss in activity occurred upon ligation of IMDQ onto the polymer backbone (which is in line with previous findings by us and others), cholesterol-polymer conjugates are still able to trigger innate immune activation.

To assess the capacity of the conjugates to induce innate immune activation *in vivo*, mice received a subcutaneous equivalent dose of IMDQ in the footpad, followed by dissection of the draining popliteal lymph node (*cfr.* **Figure S15** for a schematic of the mouse lymphoid system) and flow cytometry analysis. The latter demonstrated that both IMDQ in soluble form and when conjugated to cholesterol-polymer are capable of inducing activation of DCs (expressed by upregulation of the maturation markers CD80 and CD86), with IMDQ conjugated to cholesterol-polymer inducing a significantly higher extent of innate immune activation (**Figure 4B**). Control cholesterol-polymer that did not contain IMDQ, did not induce immune activation above the naive control. To further assess innate immune activation with a spatio-temporal resolution, we made use of an IFN-β-luciferase reporter mouse model, with a firefly luciferase gene under the control of the IFN-β promotor.[30] Type I interferons (IFN) are a major cytokine family induced by TLR7/8 triggering and potent promotors of anti-viral and anti-tumoral immune responses.[31] Bioluminescence imaging of type I IFN induction allows for non-invasive spatio-temporal evaluation of *in vivo* innate immune activation. As shown in **Figure 4C**, subcutaneous injection of IMDQ in soluble form in the footpad of mice prompts a rapid systemic inflammatory response. By contrast, cholesterol-polymer-IMDQ dramatically abrogates systemic inflammation, but by contrast focuses immune activation to the site of administration and the draining popliteal lymph node (marked by the black arrow in panel C1 of **Figure 4**), thereby confirming the flow cytometry data.

10.1002/anie.201905687

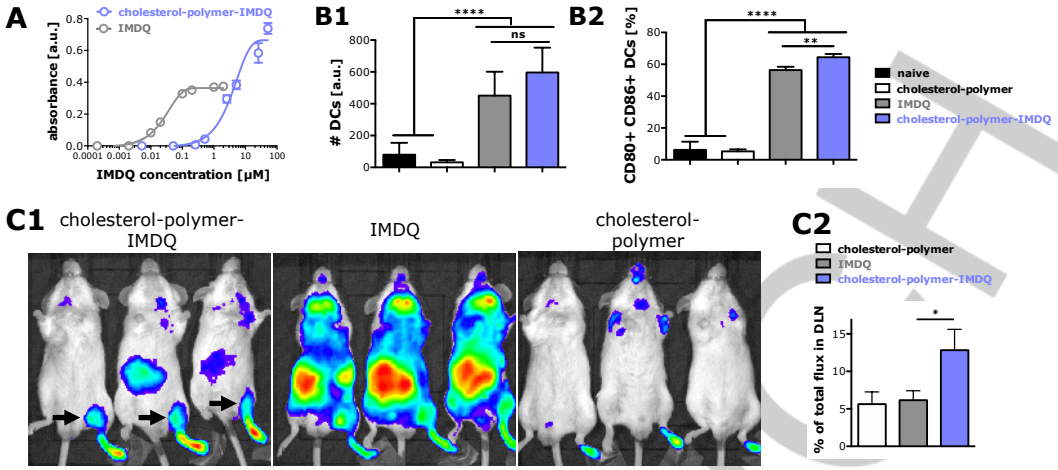


Figure 4| *In vivo* innate immune activation by lipid-polymer amphiphile conjugates. (A) TLR agonistic activity measured as NF-kB activation by a RAW-Blue reporter assay. (n =6, mean + SD). **(B)** FACS analysis of the draining popliteal lymph nodes post footpad injection (n=4, mean + SD). **(B1)** Analysis on DC count and **(B2)** activated DCs (CD80+, CD86+ cells). One-way Anova; ****:p<0,0001; **:p<0,01; n=4, mean + SD. (**C**) Bioluminescence images **(C1)** and quantification of local versus systemic response **(C2)** of luciferase reporter mice (IFNβ+/Δβ-luc), images taken 4h post footpad injection. One-way Anova; *:p<0,1; n=3, mean + SD.

In summary, we have reported on a simple yet efficient amphiphile design to deliver potent immunostimulatory small molecules to lymphoid tissue after subcutaneous administration. The amphiphile approach strongly limits systemic toxicity and could be applied to a wide variety of small molecule immunostimulatory drugs. Further ongoing design optimization focuses on positioning lipid and drug motifs at opposite polymer chain end to further increase the translational potential of the amphiphile approach.

Acknowledgements

BDG acknowledges the FWO Flanders, Kom Op Tegen Kanker and Ghent University (BOF-GOA for funding). LN is grateful for the support of the DFG (SFB 1066-B4 and Emmy Noether Program) and the Fonds der Chemischen Industrie (Liebig Program).

Keywords: lipid amphiphiles • innate immunity • immune-modulators • lymph nodes • polymers

[1] a) Q. Sun, M. Barz, B. G. De Geest, M. Diken, W. E. Hennink, F. Kiessling, T. Lammers, Y. Shi, Chem. Soc. Rev. 2019, 48, 351–381; b) L. Lybaert, K. Vermaelen, B. G. De Geest, L. Nuhn, J. Controlled Release 2018, 289, 125-124.

[2] L. Milling, Y. Zhang, D. J. Irvine, Adv. Drug Deliv. Rev. 2017, 114, 79–101.

[3] J. Hubbell, S. N. Thomas, M. Swartz, Nature 2009, 462, 449–460.

[4] A. Iwasaki, R. Medzhitov, Science 2010, 327, 291–5.

[5] S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira, C. Reis e Sousa, Science 2004, 303, 1529–31.

[6] B. J. Weigel, S. Cooley, T. DeFor, D. J. Weisdorf, A. Panoskaltsis-Mortari, W. Chen, B. R. Blazar, J. S. Miller, Am. J. Hematol. 2012, 87, 953–956.

[7] T. Y.-H. Wu, M. Singh, A. T. Miller, E. De Gregorio, F. Doro, U. D'Oro, D. A. G. Skibinski, M. L. Mbow, S. Bufali, A. E. Herman, et al., Sci. Transl. Med. 2014, 6, 263ra160.

[8] A. Z. Dudek, C. Yunis, L. I. Harrison, S. Kumar, R. Hawkinson, S. Cooley, J. P. Vasilakos, K. S. Gorski, J. S. Miller, Clin. Cancer Res. 2007, 13, 7119–7125.

[9] J. J. Moon, H. Suh, A. Bershteyn, M. T. Stephan, H. Liu, B. Huang, M. Sohail, S. Luo, S. Ho Um, H. Khant, et al., Nat Mater 2011, 10, 243–251.

[10] M. Ballester, C. Nembrini, N. Dhar, A. de Titta, C. de Piano, M. Pasquier, E. Simeoni, A. J. van der Vlies, J. D. McKinney, J. A. Hubbell, et al., Vaccine 2011, 29, 6959–6966.

[11] a) L. Nuhn, N. Vanparijs, A. De Beuckelaer, L. Lybaert, G. Verstraete, K. Deswarte, S. Lienenklaus, N. M. Shukla, A. C. Salyer, B. N. Lambrecht, et al., Proc. Natl. Acad. Sci. 2016, 113, 8098–8103; b) L. Nuhn, L. Van Hoecke, K. Deswarte, B. Schepens, Y. Li, B. N. Lambrecht, S. De Koker, S. David, X. Saelens, B. G. De Geest, Biomaterials 2018, 178, 643-651; c) L. Nuhn, S. De Koker, S. Van Lint, Z. Zhong, J. P. Catani, F. Combes, K. Deswarte, Y. Li, B. N. Lambrecht, S. Lienenklaus, N. N. Sanders, S. A. David, J. Tavernier, B. G. De Geest, Adv. Mater. 2018, 30, e1803397.

[12] G. M. Lynn, R. Laga, P. A. Darrah, A. S. Ishizuka, A. J. Balaci, A. E. Dulcey, M. Pechar, R. Pola, M. Y. Gerner, A. Yamamoto, et al., Nat. Biotechnol. 2015, 33, 1201-1210.

[13] S. Van Herck, K. Deswarte, L. Nuhn, Z. Zhong, J. P. Portela Catani, Y. Li, N. N. Sanders, S. Lienenklaus, S. De Koker, B. N. Lambrecht, et al., J. Am. Chem. Soc. 2018, 140, 14300–14307.

[14] H. Liu, K. D. Moynihan, Y. Zheng, G. L. Szeto, A. V Li, B. Huang, D. S. Van Egeren, C. Park, D. J. Irvine, Nature 2014, 507, 519–22.

[15] G. Liberale, A. Bohlok, A. Bormans, F. Bouazza, M. G. Galdon, I. El Nakadi, P. Bourgeois, V. Donckier, Eur. J. Surg. Oncol. 2018, 44, 1301–1306.

[16] C. K. Frich, F. Krüger, R. Walther, C. Domar, A. H. F. Andersen, A. Tvilum, F. Dagnæs-Hansen, P. W. Denton, M. Tolstrup, S. R. Paludan, et al., J. Control. Release 2019, 294, 298–310.

[17] Y.-R. Zheng, K. Suntharalingam, T. C. Johnstone, H. Yoo, W. Lin, J. G. Brooks, S. J. Lippard, J. Am. Chem. Soc. 2014, 136, 8790–8798.

[18] C. Wolfrum, S. Shi, K.N. Jayaprakash, M. Jayaraman, G. Wang, R.K. Pandey, K.G. Rajeev, T. Nakayama, K. Charisse, E.M. Ndungo, et al. Nat. Biotechnol. 2007, 25, 1149-1157.

[19] M. Chan, T. Hayashi, C. S. Kuy, C. S. Gray, C. C. N. Wu, M. Corr, W. Wrasidlo, H. B. Cottam, D. A. Carson, Bioconjug. Chem. 2009, 20, 1194-1200.

[20] D. Smirnov, J. J. Schmidt, J. T. Capecchi, P. D. Wightman, Vaccine 2011, 29, 5434-5442.

10.1002/anie.201905687

[21] C. Boyer, V. Bulmus, T. P. Davis, V. Ladmiral, J. Liu, S. Perrier, Chem. Rev. 2009, 109, 5402–36.
[22] A. Das, P. Theato, Chem. Rev. 2015, 116, 1434-1495.
[23] H. Willcock, R. K. O'Reilly, Polym. Chem. 2010, 1, 149–157.
[24] S. Boonyarattanakalin, S. E. Martin, S. A. Dykstra, B. R. Peterson, J. Am. Chem. Soc. 2004, 126, 16379–86.
[25] E. C. Woods, N. A. Yee, J. Shen, C. R. Bertozzi, Angew. Chem. Int. Ed. Engl. 2015, 54, 15782–8.
[26] H. Liu, B. Kwong, D. J. Irvine, Angew. Chemie Int. Ed. 2011, 50, 7052–7055.
[27] S.T. Reddy, A.J. van der Vlies, E. Simeoni, V. Angeli, G.J. Randolph, C.P. O'Neil, L.K. Lee, M.A. Swartz, J.A. Hubbell, Nat. Biotechnol. 2007, 25, 1159-1164.
[28] S. De Koker, J. Cui, N. Vanparijs, L. Albertazzi, J. Grooten, F. Caruso, B. G. De Geest, Angew. Chem. Int. Ed. Engl. 2016, 55, 1334-1339.
[29] N. M. Shukla, S. S. Malladi, C. a. Mutz, R. Balakrishna, S. a. David, J. Med. Chem. 2010, 53, 4450–4465.
[30] S. Lienenklaus, M. Cornitescu, N. Zietara, M. Łyszkiewicz, N. Gekara, J. Jabłónska, F. Edenhofer, K. Rajewsky, D. Bruder, M. Hafner, et al., J. Immunol. 2009, 183, 3229–36.
[31] L. Zitvogel, L. Galluzzi, O. Kepp, M. J. Smyth, G. Kroemer, Nat. Rev. Immunol. 2015, 15, 405–14.

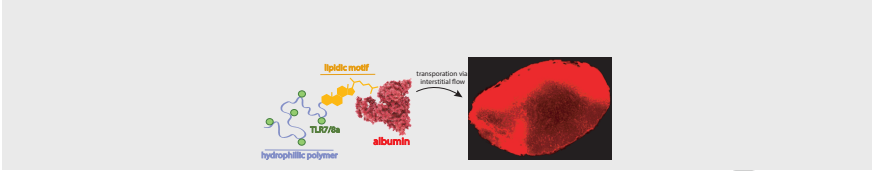
10.1002/anie.201905687

WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 2:

COMMUNICATION



Lipid-polymer amphiphile conjugates of potent small molecule immune-modulators restrict innate immune activation to lymphoid tissue

*Jana De Vrieze, Benoit Louage, Kim De Swarte, Zifu Zhong, Ruben De Coen, Simon Van Herck, Lutz Nuhn, Camilla Kaas Frich, Alexander N. Zelikin, Stefan Lienenklaus, Niek N. Sanders, Bart N. Lambrecht, Sunil A. David, Bruno G. De Geest**

Page No. – Page No.

Potent lymphatic translocation and spatial control over innate immune activation by polymer-lipid amphiphile conjugates of small molecule TLR7/8 agonists